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Reduction of Hydraulic Conductivity during Inhibition of Exudation from Excised Maize and Barley Roots[1,2]

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ABSTRACT

The uncoupler, carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP) is shown to reduce the hydraulic conductivity of barley, maize, mung bean, and onion roots. In barley and maize, the reduction in exudation from excised roots is partly due to the reduction in the permeability of the root to water (L_p), but it can be inferred that the rate of salt release to the xylem, is also inhibited. The action of CCCP on L_p is suggested to be mainly in blocking the symplasmic pathway at the plasmodesmata.

Exudation from excised roots has been explained as a standing osmotic flow in which salt release to the xylem (either active or passive) provides the osmotic pressure for water flow (1, 2). The rate of exudation from an excised root (J_v) is determined both by the rate of salt release (J_s) and by the permeability of the root to water (L_p). This interdependence of salt flow and water permeability may make it difficult to interpret effects of inhibitors on exudation from excised roots and, hence, to set up models for location of ion transport within the root. In particular, it affects the problem of whether transport from solution to the xylem involves active steps both at entry to and exit from the symplast.

Läuchli and Epstein (17) measured the effect of CCCP[3] on transfer of Cl^- across excised roots of *Zea mays* and into the exuding solution. They showed that both J_v and ^{36}Cl transport could be rapidly inhibited by about 80%. Läuchli *et al.* (18) referred to this response in support of their suggestion that active transport was required both for uptake of ions to the symplast and for release of ions to the xylem. Pitman (21) measured the effect of CCCP on ion transport across barley roots and came to the same conclusion, based on a comparison of transport of labeled ions from vacuoles in the cortex into the xylem when treated with CCCP or in a solution of negligible K^+ concentration (which also reduced net entry to the symplast). The problem of differentiating between water flow and ion fluxes was approached by also measuring transport into the shoots of intact, transpiring plants. Other studies had shown that transpiration was not inhibited by CCCP, although ion uptake could be reduced by a large percentage, depending on external salt concentration (*e.g.* refs. 14 and 19).

Baker (4) measured the effect of CCCP on distribution of $^{86}Rb/K^+$ between the cortex and stele of maize roots and on efflux from isolated cortex and stele. He found no inhibition of efflux and suggested that inhibition of transport across the root was due to action prior to the stele, *i.e.* to inhibition at the plasmalemmas of the cortical cells.

Many observations have been made showing that the permeability of plant roots to water can be reduced by inhibitors of respiration, often being detected as a reduction in transpiration. For example, Brouwer (5) gave data showing that the water conductivity of roots of bean plants could be inhibited up to 90% in KCN.

Ginsburg and Ginzburg (12) measured water flow across "sleeves" of isolated cortices of maize roots and found that L_p was inhibited 80 to 90% by 1 μM CCCP (or by 0.3 mM DNP or 16 mM KCN). The average value of L_p in control roots was quoted as $0.89 \pm 0.09 \times 10^{-7}$ cm^3 N^{-1}s^{-1} (= 89 mm^3 m^{-2} s^{-1} MPa^{-1}) which is in reasonable agreement with the values for intact roots given below (about 20 mm^3 m^{-2} s^{-1} MPa^{-1}).

Clearly, reductions in water permeability of this extent could account for the inhibition of exudation to maize roots as well as inhibition of salt transport into the xylem (J_s). A critical question is how rapidly L_p decreases in CCCP since inhibition of J_v in maize and barley can develop in a few minutes.

Here, we examine the effect of CCCP on water permeability of barley and maize roots in relation to the inhibition of exudation. The performance of the roots is compared with the expected effect of inhibition of J_s without changes in L_p.

MATERIALS AND METHODS

Seeds of barley (*Hordeum vulgare* L. cv. 'Clipper'), maize (*Z. mays* L.), or mung bean (*Vigna radiata* L. Wilczek) were germinated on aerated 0.5 mM $CaSO_4$ at 25 C in the dark. When 5 days old, the plants were transferred to aerated KCl solution in the dark containing 0.5 mM $CaSO_4$ (pH 5.5). Concentrations of KCl are quoted in the text. After 24 h in the KCl solution, the plants were used for various measurements described below. Salt bush seedlings (*Atriplex spongiosa* F.v.M.) were grown on Hoagland solution for 21 days but transferred to KCl solution (as above) for 24 h before use. Onion roots (*Allium cepa* L.) were grown from the bulbs suspended over aerated 1.0 mM KCl + 0.5 mM $CaSO_4$.

Representative data for weights of barley, maize, mung bean, salt bush, and onion roots were 120 mg/plant, 65 mg/root, 85 mg/plant, 140 mg/plant, and 75 mg/root, respectively. The specific length of barley and maize roots was 8.5 and 1.7 m g^{-1}, respectively, and the external areas were 11×10^{-3} and 5.8×10^{-3} m^2 g^{-1}, respectively. All weights of root are fresh weights and all areas refer to the external surface.

Exudation. Root exudation was measured using a graduated microscope eyepiece to observe the level of solution in a glass capillary tube. The capillary tube was fitted with silastic tubing to the root system (barley, salt bush, mung bean) or excised root (maize, onion). The roots were in aerated solutions. The rate of exudation (J_v) was calculated from a graph of volume in the tube

[1] This research was supported by the Australian Research Grants Committee.

[2] This paper is dedicated to the memory of Noe Higinbotham in appreciation of his contribution to studies of ion transport and his personal assistance and stimulation to his colleagues in Australia.

[3] Abbreviations: CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; DNP, *m*-dinitrophenol.

versus time and expressed as $mm^3\ m^{-2}\ s^{-1}$ or $mm^3\ g^{-1}\ h^{-1}$.

Hydraulic Conductivity. As described elsewhere (23), hydraulic conductivity was determined by reverse flow into mannitol solution. It is expressed here $mm^3\ m^{-2}\ s^{-1}\ Mpa^{-1}$. The reflection coefficient (σ) is not separated from L_p in these calculations, and L_p implies $\sigma\ L_p$ in what follows.

Tracer Uptake. Tracer uptake was measured using excised roots transferred from unlabeled to labeled solution for various times, then removed, and rinsed for 2 min in ice-cold 5 mM KCl + 0.5 mM $CaSO_4$, and tracer content of the dried roots was measured using a gas flow counter.

Solutions. All solutions contained 0.5 mM $CaSO_4$ and were at pH 5.5 to 6.0. CCCP was added to KCl solution as a stock solution in ethanol. To avoid osmotic changes, the same amount of ethanol was added to all solutions used for L_p or J_v measurements with CCCP. The added ethanol (1.0% v/v) had no effect on L_p or J_s over the relatively short period of the experiments, nor was there any detectable difference between roots in solutions with and without addition of ethanol.

THEORETICAL EFFECT ON INHIBITION OF J_s on J_v

The standing osmotic flow model for xylem exudation assumes that solute release to the xylem maintains a higher osmotic pressure in the vessels than in solution, so that water flows across the root at a rate proportional to L_p and the "driving force" of water potential difference. Anderson *et al.* (1) described the determination of L_p along the length of the root from knowledge of the distribution of C_x, the concentration in the xylem, and J_v with length, using the assumption of a steady state, *i.e.* $dC_x/dt = 0$ at all positions in the root.

For many systems, the distribution of C_x and J_v are not known, and J_v is determined from the volume flow from the root or root system as a whole. The concentration of the exuding fluid may be modified from that at the sites of fluid flow by subsequent water uptake or by reabsorption of solutes or further salt release. For many purposes, an "average" behavior of the root system is the only information available. Acknowledging this difficulty, however, we can make an estimate of what happens in the root by treating the xylem as a single compartment to which J_s, C_x, and L_p are uniformly distributed.

Consider what happens if the release of solute to the vessels is stopped. The rate of exudation is not necessarily reduced to zero at the same time since the solute concentration in the vessels will provide a driving force for water flow until the entry of water has diluted the xylem sap. In this case, dC_x/dt is not necessarily zero.

If L_p and the xylem volume (V_x) are constant along the length of the root, then, if Q_x is the amount of solute in the xylem:

$$\frac{dC_x}{dt} = \frac{1}{V_x}\cdot\frac{dQ_x}{dt} = \frac{1}{V_x}\cdot J_v\cdot C_x = \frac{L_p\cdot RT}{V_x}(C_x^2 - C_x\cdot C_0)$$

Hence, $C_x = C_0/(1 - Ae^{-Bt})$ where $A = (C_x' - C_0)/C_x'$, $B = \left(\frac{L_p\ RT\ C_0}{V_x}\ s^{-1}\right)$, and C_x' is the value of C_x at $t = 0$.

At time t after inhibition of J_s,

$$J_v = L_p\ RT\ C_0\frac{(A)}{e^{-Bt} - A}\ m^3\ m^{-2}\ s^{-1} \quad (1)$$

For barley roots in nutrient solution, values of V_x are 9 $mm^3\ g^{-1}$ in the outer xylem vessels or 19 $mm^3\ g^{-1}$ if the central vessel is included (or 8.2×10^{-7} and $16.4 \times 10^{-7}\ m^3\ m^{-2}$ root surface), $L_p = 15\ m^3\ m^{-2}\ s^{-1}\ MPa^{-1}$, $C_0 = 32$, $C_x' = 119.9$ mOsm, and $RT = 2426\ Pa\ m^2$. Predicted values of J_v following inhibition of J_s are calculated in Table I for the different V_x, assuming $J_v = 3.2\ mm^3\ m^{-2}\ s^{-1}$ at $t = 0$ ($121\ mm^3\ g^{-1}\ h^{-1}$).

Alternatively, if L_p changes during the inhibition, the values of J_v can be used to estimate L_p, although substitution in equation 1 is not valid. After a short period, Δt (about 60 s), the amount of solute initially in volume V_x is distributed in volume $V_x + J_v\cdot\Delta t$, and a new value of C_x can be calculated. Hence, L_p can be calculated from J_v over the period t to $(t + \Delta t)$ to obtain a good approximation to the change in values of L_p that is at least as accurate as the experimental determination. (Fig. 7).

Table I. *Values of J_v following Inhibition of J_s*

The calculations were for barley roots, assuming V_x is (*a*) all the xylem and (*b*) only the metaxylem vessels.

Time	J_v	
	$V_x = 19\ mm^3$	$V_x = 9\ mm^3$
s	$mm^3\ m^{-2}\ s^{-1}$	
0	3.2	3.2
60	2.7	2.4
120	2.4	1.9
300	1.7	1.1
600	1.1	0.53
1,200	0.52	0.26

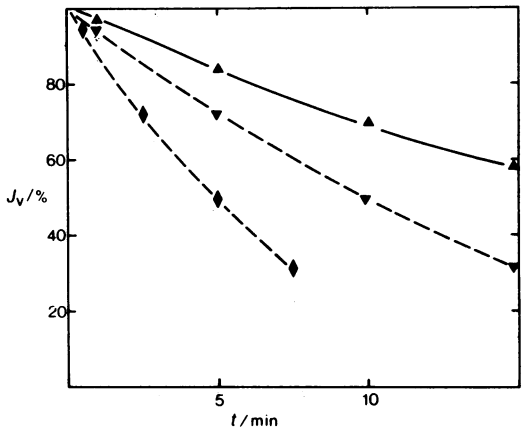

FIG. 1. Calculated percentage decrease in J_v using data from Table II or the equation assuming L_p is constant (▲——▲). Similar estimates of J_v were made using iterative calculations with V_x taken as 28 $mm^3\ m^{-1}$ ($8.284 \times 10^{-6}\ m^3\ m^{-2}$) (▼- - -▼) or with reduction in V_x to 14 $mm^3\ m^{-1}$ (◆- - -◆), which increases the rate of adjustment of J_v.

In practice, J_s may include components proportional to water flow and, perhaps, external concentration. In this case, the inhibition of J_v will develop less rapidly than that given in Table I (or in Fig. 1). The contribution of salt release to the xylem following inhibition of active transport components could be estimated from the decrease in J_v if V_x were known and L_p were determined separately during the inhibition. Then C_x can be calculated (Table IV) and, hence, dC/dt so that

$$J_s = V_x\cdot\frac{dC_x}{dt} + J_v\cdot C_x \quad (2)$$

Data from (1) showed that L_p was not uniform along maize roots and the above approach then has limitations. More appropriately, the effect of inhibition of J_s can be estimated using an iterative computing technique in which L_p is assumed constant over short lengths of root and input/output of water and solutes computed for this segment and for short time intervals.

The procedure is as follows, using concentrations C_1 and C_2 at distances separated by Δx at time t and C_1' and C_2' at time $t + \Delta t$. Volume flow was J_1 and $J_2\ m^3\ s^{-1}$ at time t and J_1' and J_2' at time $t + \Delta t$.

1. Given C_1 at t, enter C_2 and calculate mean concentration as $\bar{C} = (C_1 + C_2)/2$.

2. Calculate mass transfer at plane 1 as $(C_1 \cdot J_1 \Delta t)$ where J_1 is known from the previous cycle.

3. Calculate J_2 and J_1 + osmotic water flow, *i.e.* $J_2 = J_1 + L_p \cdot RT \cdot \Delta x \cdot a\ (\bar{C} - C_0)$ $m^3\ s^{-1}$ where a is the specific area at the surface ($m^2\ m^{-1}$); hence, mass transfer is $C_2 J_2 \Delta t$ and $\Delta \bar{C} = (C_1 J_1 - C_2 J_2)\ \Delta t/(\Delta x \cdot V)$, where V is a specific xylem volume ($m^3\ m^{-1}$).

4. From $\bar{C}$, C_2' can be calculated, given C_1'.

Table II illustrates the variation in L_p and C_x that has been found for maize roots by Anderson *et al.* (1). These values are used here to compare with the situation where L_p is assumed independent of x. They form the basis for the calculated decrease in J_v with t shown in Fig. 1; σ has been taken as 1.0. For comparison, the simple calculation based on equation 1 is given, using average values for 5 cm root of L_p = 21.6 $mm^3\ m^{-2}\ s^{-1}\ MPa^{-1}$, $V_x = 8.284 \times 10^{-6}\ m^3\ m^{-2}$, and C_x = 45 mosm. When L_p changes along the length of the root, as in Table II, the simpler average calculation does not agree with the iterative calculations, although the simpler calculation is valid when L_p is set constant along the root.

As with barley, there is some uncertainty about the volume of xylem in the maize root. Measurements from photographs show that the xylem volume is about 28 $mm^3\ m^{-1}$ root, but House and Findlay (15) found that the time course of osmotic equilibration was better explained if the effective volume were about 50% of the xylem volume in roots 5 to 7 cm long. The smaller volume has the effect of allowing J_v to fall more rapidly when J_s is inhibited (Fig. 1) and of changing the time scale proportionately to the reduction in volume.

RESULTS

Barley. Substantial inhibition of J_v was produced by CCCP concentrations above 1 μM but was most rapid at 60 μM (Fig. 2). There was no indication of stimulation of J_v at lower concentrations, as found for maize cortices (12).

Processes that could contribute to the reduction in J_v are a decrease in L_p and an inhibition of J_s. The change in L_p can be determined by reverse flow to mannitol or to mannitol + CCCP, after various times in CCCP. For each root, a measurement of L_p was made using 5 mM KCl and 200 mM mannitol + 5 mM KCl (23). Twenty to 30 min after return from mannitol to KCl solution, J_v had recovered to a value close to that before transfer to mannitol. The root then was transferred to CCCP for varied times, and L_p was re-determined. Repeat measurements of L_p with the same root in 5 mM KCl showed that this first estimate of L_p did not affect subsequent measurements of J_v and L_p (Table III; Fig. 3). In 60 μM CCCP, there was a rapid reduction of L_p to about 20% of that in 5 mM KCl. It was difficult to estimate L_p at shorter exposures to CCCP due to the time taken for reverse flow to reach its steady value for long enough to measure (about 3–4 min). When transferred directly from KCl to mannitol + 60 μM CCCP, the reverse flow was reduced by 50% in about 3 min, falling to 33% in 5 min. These values support the suggestion that L_p was reduced extremely rapidly in these roots after transfer to 60 μM CCCP. In 1.25 μM CCCP, the reduction in L_p took much longer to develop (Fig. 4).

Table II. *Data for L_p and C_x*

The data were from Anderson *et al.* (1) for L_p and C_x in maize roots in 1 mM KCl.

Length	L_p	C_x
cm	*$mm^3\ m^{-2}\ s^{-1}\ MPa^{-1}$*	*mosm*
1	122	8.5
2	80	15.5
3	46	23.5
4	29	31.5
5	24	40
6	25	46.5
7	28	45.5

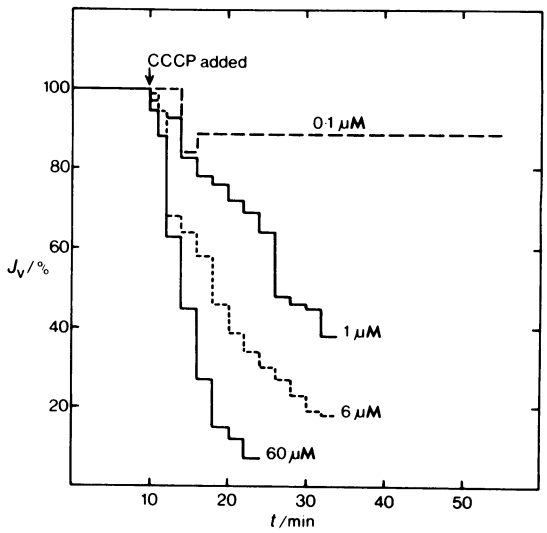

FIG. 2. Rates of exudation expressed as percentage of the rate in the 10 min prior to addition of CCCP. Ethanol (1% v/v) was present in all solutions. Initial rates (in $mm^3\ g^{-1}\ h^{-1}$) were: 60 μM, 40; 6 μM, 34; 1 μM, 18; 0.1 μM, 18.

Table III. *Effect of CCCP on L_p in Barley Roots*

Pairs of estimates on the same barley root system were determined by reverse flow to 200 mM mannitol solution. Mean J_v in KCl = 2.25 $mm^3\ m^{-2}\ s^{-1}$.

Time in CCCP	L_p	
	In solution	In KCl + 60 μM CCCP
min	*$mm^3\ m^{-2}\ s^{-1}\ MPa^{-1}$*	
0	15	18
	15	16
	10	8
5	15	3.3
		2.1
7	15	2.3
	15	2.3
8	15	3.2
18		2.7
30	9	1.5
	21	1.9
120	9	1.7
160	12	1.3

As outlined in "Materials and Methods", the decrease in exudation following addition of CCCP depends both on the rate of decrease of J_s and on the rate at which the osmotic content of the vessels becomes diluted. Using data for J_v and L_p, it would be possible to calculate the average values of C_x in the root as $J_v = L_p\ (C_x - C_0)$. Using the relation between J_s and C_x given above (equation 2), J_s then can be estimated. One experimental difficulty is that separate roots have to be used for each estimate of L_p, introducing more variability than is seen in measurement of J_v from a single root. However, the graph of L_p (as a percentage of control) *versus* J_v (as a percentage of untreated rate) shows less variability (Fig. 3), and such a graph can be used to obtain values for L_p appropriate to the different values for J_v during inhibition in a single root. This procedure has been followed to obtain the curves in Figure 4 and to calculate C_x and J_s (Table IV), assuming V_x for barley is $8.2 \times 10^{-7}\ m^3\ m^{-2}$ root surface. Inhibition of J_s

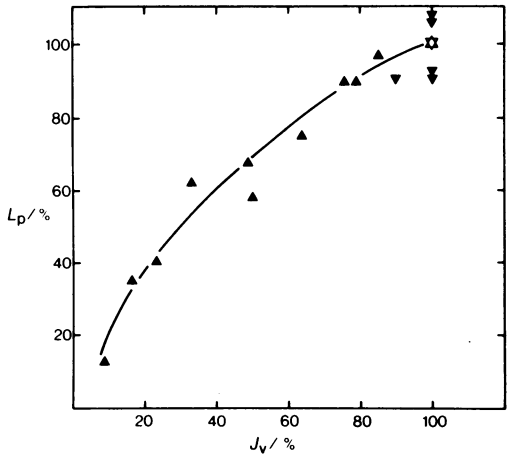

FIG. 3. Estimates of L_p after varied times in 1.25 μM CCCP plotted against the corresponding values of J_v, both expressed as percentages of the control values for each barley root system. Mean values for 100% were J_v = 1.50 mm^3 m^{-2} s^{-1} and L_p = 5.13 mm^3 m^{-2} s^{-1} MPa^{-1}.

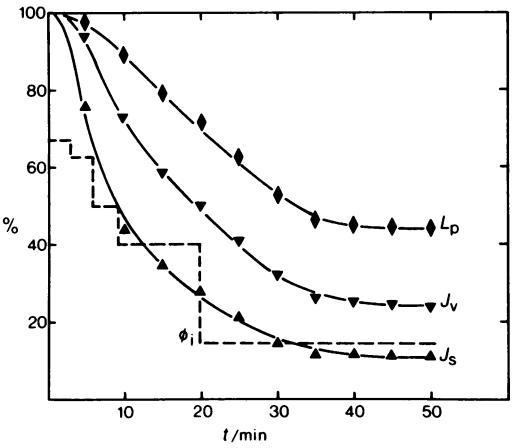

FIG. 4. Data were collected from barley roots in 1.25 μM CCCP for ϕ_i (- - -; 100% = 60 nmol m^{-2} s^{-1}), J_v (▼——▼; 100% = 1.56 mm^2 m^{-2} s^{-1}), and L_p (◆——◆); 100% = 6.28 mm^3 m^{-2} s^{-1} MPa^{-1}). Values were estimated for J_s (▲——▲; 175 nmol m^{-2} s^{-1}).

Table IV. *Estimated Effect of CCCP on J_s in Barley Roots*

Calculation of J_s from data for J_v and L_p was for barley roots in 5 mM KCl with 1.25 μM CCCP.

Time in CCCP	J_v	L_p	C_x	$J_v \cdot C_x$	$V_x \cdot dC_x/dt$	J_s
min	*mm^3 m^{-2} s^{-1}*	*mm^3 m^{-2} s^{-1} MPa^{-1}*	*mosm*	*nmol m^{-2} s^{-1}*		
0	1.56	6.28	113	173	0	175
5	1.47	6.15	109	160	25	135
10	1.14	5.59	95	108	30	78
20	0.78	4.52	82	64	15	49
30	0.50	3.33	72	36	9	27
40	0.39	2.83	67	26	5	21

appears to develop more rapidly than the reduction in L_p, falling to 50% in about 10 min.

Uptake of ions to the root was measured using ^{36}Cl as a tracer. From a graph of tracer content plotted against the time after addition of tracer and CCCP, it was possible to calculate the net tracer influx (ϕ_i). Figure 4 shows the following combined responses to 1.25 μM CCCP: (*a*) ϕ_i determined from uptake of ^{36}Cl; (*b*) J_v determined from volume exuded (from Fig. 3); (*c*) L_p determined by reverse flow to mannitol (from Fig. 3); (*d*) J_s calculated from J_v and L_v (Table IV and Fig. 3). Figure 5 gives similar data for ϕ_i, J_v, and L_p for roots put into 60 μM CCCP and includes calculated values of J_v, assuming that J_s becomes zero at t = 0, using the two values for V_x (equation 1).

In both concentrations of CCCP, inhibition of ϕ_i occurred before the decrease in J_v, although the rapid response of the roots in 60 μM CCCP makes the difference less evident than at the lower concentration. There was direct evidence (Table III) that L_p decreased in CCCP, although, at 60 μM CCCP, the reduction was so rapid that L_p had fallen to about 20% at the earliest measurement. In Figure 5, the decrease in J_v was more rapid than predicted, due presumably to the decrease in L_p. Where J_s could be estimated (Fig. 4), it decreased more rapidly than the change in L_p. At 20 min, J_s had been inhibited 55% when L_p had been reduced only about 15%. Whatever the mechanism involved, it seems that CCCP both inhibits J_s and reduced L_p in barley roots.

It has been suggested (3) that streaming of cytoplasm is important in transport of ions across the root to the stele. It was observed with barley roots that streaming was rapidly inhibited to 60 μM CCCP. In 1.25 μM CCCP, it had fallen by about 60% in 10 min.

Maize. A number of studies have been made of the effects of inhibitors on uptake of ions to maize roots and to maize root cortices (6, 18). Figure 6 shows the development of inhibition of J_v and ϕ_i in maize, as is shown in Figure 5 for barley. Uptake of ^{36}Cl was inhibited extremely rapidly by 60 μM CCCP; over the first 2 min, the average rate fell to 16% of that in controls. There was a lag of 1 to 2 min before J_v showed any inhibition, but then it fell more rapidly than predicted from the calculations already described (Table 1) even when V_x was taken as 14 mm^3 m^{-1} or 50% of the actual xylem volume (15).

These estimates of L_p are averages for the whole root, although data from reference 1 show that L_p changes along the length. As CCCP produces an 80 to 90% reduction in L_p, it is likely to be having a general effect on L_p. If exudation from the root is represented by the simpler model using average values of L_p and C_x, then changes in L_p can be estimated from the decrease in J_v, if it is assumed that J_s is inhibited by CCCP at least as rapidly as ϕ_i. These calculations are shown in Figure 7 for roots in 1 and 10 mM KCl, using V_x = 28 mm^3 m^{-1} (using V_x = 12 mm^3 m^{-1} changed L_p by only 10% in the middle of its range, so the calculation is not particularly sensitive to V_x). The figure includes experimental estimates of L_p determined by reverse flow to mannitol, which are in reasonable agreement with the decrease in L_p

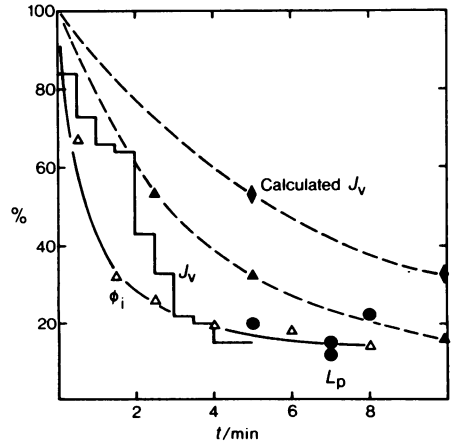

FIG. 5. Effect of 60 μM CCCP on rate of ^{36}Cl uptake ϕ_i (△——△) J_v (——), L_p (●), and calculated values of J_v, assuming V_x = 19 mm^3 (◆- - -◆) or 9 mm^3 (▲- - -▲). Values at 100% were: ϕ_i = 50.8 nmol m^{-2} s^{-1}; J_v = 2.25 mm^3 m^{-2} s^{-1}; L_p = 13.3 mm^3 m^{-2} s^{-1} MPa^{-1}.

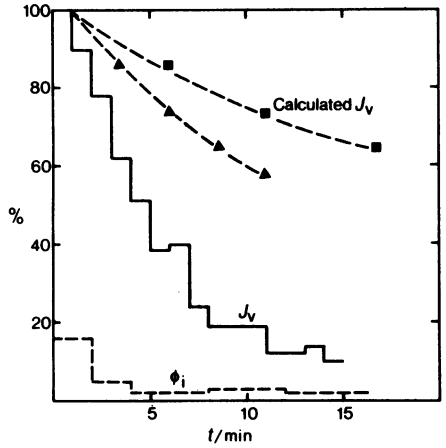

FIG. 6. Exudation from maize roots in 1 mM KCl + 0.5 mM $CaSO_4$ with 60 μM CCCP added at $t = 0$. Uptake of ^{36}Cl (– – –; 100% = 1.2 nmol m^{-2} s^{-1}. Exudation, J_v (——; 100% = 3.0 mm^3 m^{-2} s^{-1}). Values of J_v were calculated, assuming V_x = 14 mm^3 m^{-1} (▲) or 28 mm^3 m^{-1} (■) (see Fig. 1).

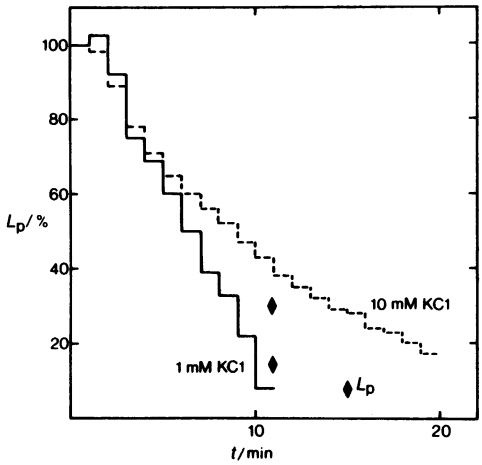

FIG. 7. Calculated values of L_p after addition of 60 μM CCCP at $t = 0$ to maize roots in 1 or 10 mM KCl (+0.5 mM $CaSO_4$) and experimental values (◆); see text for procedure.

calculated on the assumption that J_s is inhibited. These results support the view that hydraulic conductivity can be reduced rapidly by CCCP.

Comparison with Other Species. Figure 8 gives a comparison of the effect of 60 μM CCCP on the rate of exudation, J_v from roots of several plant species. These are representative examples selected from a number of different experiments. Previous work (5, 12) has shown that various inhibitors of respiration reduce L_p in beans as well as in maize roots. In Figure 8, both mung beans and onion roots show inhibition of J_v, although it develops more slowly than in maize or barley. Roots of saltbush at first showed no inhibition, but an increased exudation which then fell after 30 min. It is thought that the increase is due to increased efflux from cells of the root into the xylem, but this interpretation needs further investigation.

Table V gives the values of L_p in mung bean and onion roots, showing that it had fallen to about 15% after 2 to 3 h in CCCP. Estimation of L_p from reverse flow to mannitol in *Atriplex* roots gave extremely small values in the untreated roots, and it was assumed that this was due to osmotic shock produced by the mannitol solution (16).

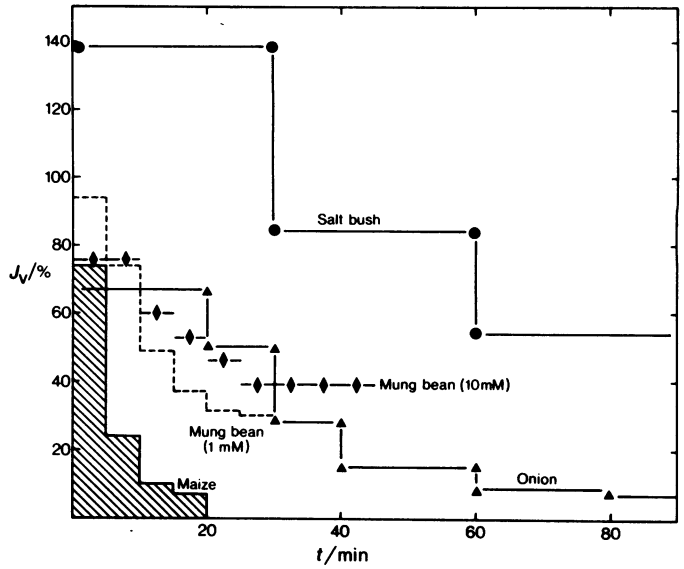

FIG. 8. Rates of exudation from roots of several plant species expressed as a percentage of control values. CCCP (60 μM) was added at $t = 0$. Maize, from data of Figure 5; onion in 1 mM KCl (▲——▲), 100% = 2.1 mm^3 m^{-2} s^{-1}); mung bean in 1 mM KCl (– – –; 100% = 1.2 mm^3 m^{-2} s^{-1}; and in 10 mM KCl (◆——◆; 100% = 0.8 mm^3 m^{-2} s^{-1}); salt bush in 1 mM KCl (●——●; 100% = 0.7 mm^3 m^{-2} s^{-1}).

Table V. *Effect of CCCP on L_p in Mung Bean and Onion Roots*

Values of L_p were determined by reverse flow in mung bean and onion roots after various times in 60 μM CCCP.

Root	L_p in KCl	Time in CCCP	L_p in CCCP	Control
	mm^3 m^{-2} s^{-1} MPa^{-1}	*min*	mm^3 m^{-2} s^{-1} MPa^{-1}	%
Mung bean	8.5	10	3.4	40
	5.6	10	4.4	78
	5.6	30	1.9	34
	5.6	60	2.3	41
	5.6	85	2.9	52
	4.4	180	0.8	18
	4.5	190	0.6	13
Onion	27	220	2.0	8
	12.2	120	1.5	12

DISCUSSION

The data presented in this paper show that, in maize and barley, the hydraulic conductivity can be reduced to 10 to 20% of the control value within 5 min (barley) or 10 to 15 min (maize) by the inhibitor CCCP. At lower concentrations of CCCP, it was possible to infer that there was also an inhibition of J_s. These results raise questions about the action of CCCP in reducing L_p and the effect of these observations on suggestions that ion release to the xylem involves active transport.

Action of CCCP. CCCP has several effects on cell metabolism arising from its effect on proton permeation of the membranes. One is the well-known action as an uncoupler of oxidative phosphorylation leading to reduction in ATP level. Another action is an increase in proton permeability of the plasmalemma. Inhibition of ion transport into the cell could result either from the low level of ATP or from the release of proton gradients between solution and the cytoplasm. In *Riccia fluitans*, both effects have been demonstrated (11), whereas rapid decreases in ATP in barley roots in CCCP have been found. There may also be secondary effects of CCCP which could affect transport in the symplast, such as

inhibition of cytoplasmic streaming and permeability of plasmodesmata.

Various studies (5, 12) have shown that a reduction in hydraulic conductivity of roots can be produced by various inhibitors of respiration (*e.g.* KCN, DNP, arsenite) and there is evidence from Table V and Figure 8 as well as from various publications (5) that this response can be found in plants of many species. The reduction in hydraulic conductivity should also be detected from the reduction in leaf water potential of transpiring plants treated with these inhibitors.

It is difficult to see how CCCP or these other inhibitors could affect water transport in the cell walls and intercellular spaces. Within the symplast, however, there are various ways that CCCP could act on water flow; for example, on entry across cell membranes, on transport through plasmodesmata, or on cytoplasmic streaming.

Drake (9) has shown that azide and cyanide both reduce the electrical coupling between parenchyma cells of the oat coleoptile, implying that the electrical resistance of the plasmodesmata is increased by this treatment. Drake suggests that, with KCN, deposition of callose may be responsible, as discussed elsewhere (10). Other explanations were not completely ruled out since azide also reduced electrical coupling but did not produce any visible sign of callose. We have examined barley and onion roots in which L_p was reduced to about 15% by CCCP and found no visible evidence of callose deposits over pit fields. Drake (9) also found that the reduction in coupling recovered very slowly (many hours) and we have found that reduction in L_p, too, is not readily reversible. Whatever the mechanism of the changes in electrical resistance, the observations are valuable for their implication of plasmodesmata and symplasmic transport in the reduction of L_p.

There are reports in the literature that CCCP reduces the hydraulic conductivity of cell membranes [discussed by Dainty (7)]. Wooley (28) measured the time course of ^3H_2O exchange in maize roots pretreated with CCCP for 1 h. The time for 50% exchange increased from 26 s in normal to 150 s in treated roots, implying a reduction in bulk diffusion coefficient. Jarvis and House (16) also measured ^3H_2O exchange in maize roots but, as with Wooley's measurements, it is not possible to distinguish whether the apoplast or symplast is the pathway of exchange. If diffusion in the symplast were blocked at the plasmodesmata by CCCP, then this could produce the observed decrease in rate of isotopic exchange by restricting exchange to the apoplast. Glinka and Reinhold (13) measured ^3H_2O exchange in cylinders of carrot tissue and found no effect of CCCP at 3 and 10 μM, although the method was capable of detecting changes in L_p due to AbA. It seems more likely that this method would measure conductance of the membranes rather than internal conductance of the symplast. However, there is another observation with storage tissue (25) showing that DNP reduced water loss from potato tuber slices but, as with roots, it is not clear whether this is a membrane or symplast response. Although CCCP may reduce hydraulic conductivity of the cell membranes, the distinction between the membrane and symplast in plant roots does not seem to have been established, and there is good reason to think that these inhibitors are reducing the conductivity of the symplast quite apart from any effect they may have on the membranes.

The importance of cytoplasmic streaming in water transport across the root has been discussed (26), but it is difficult to assess its importance and it is more likely to affect solute transport than water flow. In the roots studied, CCCP inhibited streaming and reduced ATP levels at 60 μM, but it had a less rapid effect at 1.25 μM on streaming. In other studies using arsenite as an inhibitor, it has been shown that inhibition of J_v and reduction in L_p can occur without stopping cytoplasmic streaming (unpublished data).

The relative importance of the symplast and apoplast in transport across the root has been a matter of much discussion. Molz and Ikenberry (20) considered that about 30 to 50% of the flow was in the apoplast. Estimation of the permeability of plasmodesmata (24) shows that a much higher proportion could flow in the symplast than the apoplast and, in any case, passage across the endodermis should involve the symplast since the casparian strip blocks the apoplast at the endodermis. It is likely, too, that the proportion transported in the symplast changes with water flow since L_p has been found to increase with water flow in many plants (27). Irrespective of how CCCP affects L_p, the 80 to 90% reduction found with CCCP shows that the pathway through cell membranes, cytoplasm, and symplast could account for about 80% of water transport at the low flow rates of exudation.

Does CCCP Inhibit Release to Xylem? The results given above show clearly that the reduction in exudation and, hence, in salt transport from excised roots is due partly to a reduction in L_p, but there is also evidence that J_s is reduced. What is not clear is whether the reduction in J_s is due to an effect on release to the xylem or on transport in the symplast across the root. It is likely that CCCP could affect both processes by reduction in ATP content and by the blocking of the plasmodesmata. Better evidence for active secretion to the xylem comes from the electrophysiological studies of Davis and Higinbotham (8) and the response of ion transport in excised roots and intact plants to AbA (23) and azetidine 2-carboxylic acid (a proline analog) (22).

However, the comparison of other species shows that CCCP can increase exudation in *A. spongiosa*, and this appears to be due to increased ion release to the xylem. Davis and Higinbotham (8) also found evidence for active Cl^- transport from the xylem to the stele and a more useful view of the stele-xylem interface may be one in which both active influx and efflux are involved in regulation of the amounts of ions released to the xylem. Reabsorption from the xylem to the stele could be particularly important to plants growing under saline conditions, as with salt bush.

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